Mobilization and Collection of Peripheral Blood Stem Cells in Patients with Sickle Cell Disease

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A. SPECIFIC AIMS/ OBJECTIVES

Primary Objective:

1. To collect mobilized peripheral blood stem cell (PBSC) from patients with Sickle Cell Disease (SCD) for use in research studies.

Secondary Objectives:

1. To examine mobilization kinetics of peripheral blood stem cells after plerixafor in patients with SCD.
2. To examine apheresis techniques to optimize PBSC collections in SCD.

B. BACKGROUND AND SIGNIFICANCE

Sickle cell disease and treatment options
SCD is one of the most common genetic diseases in the world with an estimated 2600 babies born with SCD each year[1] and approximately 70,000 to 100,000 individuals of all ages affected in the United States[2]. The clinical manifestations of SCD include acute events such as recurrent debilitating painful crises, as well as life-threatening pulmonary, cardiovascular, renal, and neurologic complications. The only established curative treatment for SCD patients is allogeneic hematopoietic stem cell transplant (HSCT). Unfortunately, access to this intervention is limited by availability of suitable matched donors and HSCT is associated with significant morbidity and mortality. For patients who cannot undergo HSCT, treatment of SCD has been limited primarily to hydroxyurea and supportive symptomatic care. After decades with very few novel therapeutic options for SCD patients, autologous cell-based genetic therapies, including lentiviral-based gene therapy as well as gene editing, now offer the possibility of innovative curative approaches for patients lacking a matched donor for hematopoietic stem cell transplantation.

Genetic therapies in sickle cell disease
Gene therapy for sickle cell disease is increasingly promising and there are currently open clinical trials at several centers that employ gene addition or gene editing strategies. Here at Boston Children's Hospital, extensive laboratory investigation has led to the development of a unique strategy for achieving fetal hemoglobin induction through gene therapy. Specifically, downregulation of the transcription factor BCL11A by shRNA leads to potent derepression of fetal beta-globin chain and elevated levels of fetal hemoglobin (HbF)[3]. A lentiviral vector expressing a potent anti-BCL11A shRNA^{miR} has been designed and undergone extensive preclinical testing [4,5], and a clinical protocol has been open for a Phase I/II clinical trial testing this vector in patients with sickle cell disease (Phase I/II Pilot and Feasibility Study of Hematopoietic Stem Cell Gene Transfer for Sickle Cell Disease). To date, 6 patients have been enrolled after successfully collecting with plerixafor and manipulating their PBSC in the laboratory.

Sufficient CD34+ cell procurement is critical to the success of gene therapy
Novel genetic therapies, including gene therapy, rely on safely and effectively obtaining an adequate yield of autologous hematopoietic stem and progenitor cells (HSPCs) for genetic modification and transplantation. HSPCs are generally identified within cell fractions enriched for the expression of the CD34 marker. The minimal cell dose is unknown for the hemoglobinopathy setting because in these disorders the genetically-corrected stem cells likely

lack a selective advantage, so a graft with low CD34+ cell number would be more likely to fail. For ensuring proper rescue of hematopoiesis after fully myeloablative conditioning, a minimum range of CD34+ cells is 2-5 x 10^6 cells/kg. Gene therapy product administration may also require a freezing and thawing process which could further affect the cell dose at infusion. Therefore, the standard minimum recommended dose for manipulated cells is 5 x 10^6 CD34+ cells/kg. Importantly, cell dose requirements in gene therapy also foresee that a fraction of the harvest will be stored as un-manipulated back-up for safety reasons requiring an additional minimum dose of 2 x 10^6 CD34+ cells/kg. Therefore, the estimated number of cells required for collection is about 10 x 10^6 CD34+ cells/kg if we include a backup and allow for cell loss during processing.

Obtaining adequate CD34+ cell yield by bone marrow harvest is challenging in patients with SCD

Options for autologous HSC collection include bone marrow harvest or peripheral blood HSC mobilization. Bone marrow (BM) harvest is an invasive procedure requiring anesthesia, which is associated with sickle cell-related morbidities, and may not achieve goal CD34+ cell dose, necessitating repeated procedures scheduled over multiple months. Initial data from the bluebird bio (bbb) HGB-206 LentiGlobin gene therapy trial indicates use of only CD34+ cell doses of 2.7 and 2.9 x 10^6 cells/kg in the first two patients[6]. In bbb's experience, most subjects have required more than one bone marrow harvest to achieve a minimum cell dose of 1.5 x 10^6 CD34+ cells/kg, with some subjects requiring up to 4 harvests, and unfortunately, the harvest procedures have been associated with hospitalization for sickle cell crises.

In most gene therapy trials, rather than bone marrow harvest, HSCs are obtained through peripheral collection after mobilization with granulocyte colony-stimulating factor (G-CSF) followed by peripheral blood (PB) apheresis. However, this approach is contraindicated in SCD because G-CSF has been reported to cause severe adverse effects in sickle cell patients[7]. Even with doses sometimes smaller than standard, G-CSF has been shown to result in vaso occlusive crises, severe acute chest syndrome[8], and in one report, massive splenomegaly and death[9]. A small study[10] illustrated efforts to collect HSCs from SCD patients without mobilization agents. For example, by withdrawing hydroxurea from patients who had taken it chronically, numbers of circulating progenitors did increase 12 -14 days after hydroxyrea withdrawal, however not in sufficient numbers to allow for efficient HSC collection. Additionally, in the same report, one sickle cell patient underwent leukapheresis without any mobilization agent, and unsurprisingly, the yield was unacceptably low. Alternative options for mobilization are needed.

Plerixafor background and safety

Plerixafor is a mobilization agent that causes HSC mobilization by reversibly inhibiting the binding of the chemokine stromal-derived factor 1 (SDF-1/CXCL12) to its receptor CXCR4, which is expressed on the surface of HSCs. Disruption of the SDF-1/CXCL4 interaction results in the rapid release of HSCs from the bone marrow[11]. Short-term administration of plerixafor is safe and well-tolerated in healthy volunteers[12], healthy sibling donors[13], and when combined with GCSF in patients with lymphoma and multiple myeloma[14-16]. It was FDA approved in 2008 for use in combination with GCSF in patients with multiple myeloma and non-Hodgkin lymphoma. Plerixafor is also increasingly being used in the gene therapy setting. In clinical trials of gene therapy for Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), and adrenoleukodystrophy (ALD), plerixafor mobilization has been well-tolerated and resulted in good cell procurement[24].

With respect to duration of activity, plerixafor is a very short-acting medication. Plerixafor (then known as AMD-3100) was first investigated as an antiretroviral agent with potential benefit for

HIV patients. In a study of safety and pharmacokinetics in 12 healthy volunteers[17], subjects received AMD-3100 at doses of 10 – 80 mcg/kg (lower than the current FDA-approved dose of 240 mcg/kg). Peak drug concentration in blood occurred at 0.25 – 1.2 hours), and the peak WBC count was observed at 6 hours after infusion, with return to baseline WBC count at 24 hours. The median elimination half-life was 3.6 hours, with essentially undetectable blood levels by 12 hours. A subsequent PK study in which HIV patients were treated with doses up to 160 mcg/kg demonstrated a mean half-life of 8.6 hours[18]. Finally, in another 2003 study aimed at exploring AMD-3100 as a stem cell mobilizing agent, Liles et al administered doses of 40 – 240 mcg/kg to healthy subjects and reported a peak increase in WBC count at 6-9 hours[12]. Plerixafor is now under investigation for use as a single agent in normal healthy volunteers and in donors for allogeneic stem cell transplants, with minimal side effects after administration[22].

Adverse effects associated with plerixafor have been mild and transient, including headache, erythema and stinging at injection site, perioral paresthesias, nausea, and sensation of abdominal distension, all of which resolved within 24 hours after drug administration in the early trials. Hematologic effects observed have included leukocytosis (although less than with G-CSF) and transient thrombocytopenia[12]. Splenic enlargement was observed following *prolonged* (2-4 weeks) daily plerixafor administrations in rats at doses approximately 4-fold higher than the recommended human dose (drug label information). The most common adverse reactions (>/= 10%) reported in patients who received plerixafor in conjunction with G-CSF regardless of causality and more frequent with plerixafor than placebo during HSC mobilization and apheresis were diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting (drug label information). The FDA-approved dose for plerixafor (in combination with G-CSF) for stem cell mobilization in patients with multiple myeloma or non-Hodgkin lymphoma is 0.24 mg/kg, with a maximum recommended dose of 40 mg.

Plerixafor in sickle cell disease
Plerixafor has been compared to G-CSF in a sickle cell mouse model, and results showed effective mobilization of HSC subsets, without neutrophil or endothelial activation, and with lower total WBC and neutrophil counts compared to G-CSF-treated mice[20]. This in part led to three trials to date that have published the safety and/or efficacy of mobilizing PBSC in SCD patients with plerixafor[25-27], including one performed here at BCH[25]. Plerixafor was associated with an earlier mobilization (2-4 hours) and successful collection of stem cells. There was no evidence of leukostasis, splenic enlargement or thrombocytopenia due to plerixafor. All SAE's in our study at BCH were not directly related to plerixafor administration nor apheresis. Presently, plerixafor is the standard agent used at the FDA recommended dose of 0.24 mg/kg for mobilization in trials for gene therapy in SCD.

Apheresis for Stem Cell Collection in SCD
PBSCs are collected via continuous-flow apheresis in a manner similar to that used to collect platelets from healthy donors that has been used in clinical practice since the early 1980s[23]. PBSC transplant was only accepted as a standard clinical modality once strategies were developed to increase the stem cell yield including apheresis following chemotherapy and/or the administration of hematopoietic growth factors. Apheresis uses centrifugal technology, which has been validated for donors with healthy red blood cells. Part of our study will examine the apheresis instrument settings that can improve collection efficiency of the process. Changing these settings would have no effect to the donor however they may increase or decrease the cell yield.

From our previous experience with plerixafor mobilization and collection, we found no significant SAE's in volunteers as we had decreased the risk of adverse sickling reactions by selecting those volunteers who participate in an exchange transfusion program or a chronic transfusion program prior to plerixafor administration. This procedure is a common component of sickle cell care and will decrease the subject's baseline levels of HbS to below 40% with a concomitant reduction in the risk of any vaso-occlusion events. Simple or exchange transfusions are commonly used in SCD patients in the pre-operative period to prevent the development of VOC, acute chest syndrome, or stroke after surgery. Patients who receive preoperative transfusions have a decreased rate of complications[21]. Exchange transfusions are routinely used preoperatively, for treatment of acute stroke, and for treatment of acute chest syndrome.

Subsequent use of PBSC in Research Studies:
The goal of this study is to collect PBSCs from SCD adult volunteers. Investigators at Boston Children's Hospital, and other collaborating institutions or companies, and the NIH as part of the Cure Sickle Cell initiative will use the PBSCs in laboratory investigation of methods to purify SCD stem cells, isolate, manipulate using experimental methods and study subpopulations of cells. The studies that will be performed will evaluate hematopoeisis, gene transfer and gene editing techniques and the impact of cryopreservation and reconstitution on HSCs. These studies are necessary to develop standard methodologies as part of the required preclinical data for FDA and NIH submissions for future human gene transfer studies.

C. DESIGN AND METHODS

(1) Design

This is a study in which we will collect peripheral HSPCs from SCD patients by apheresis. Subjects will be administered the standard plerixafor mobilization dose of 240 mcg/kg and hydrated in the hospital overnight, with apheresis performed for 3-4 blood volumes. Patients will be observed to ensure that they tolerated their apheresis collection without adverse events and discharged.

(2) Patient Selection and Inclusion/Exclusion Criteria

Subjects will be enrolled following confirmation of eligibility criteria. Informed consent will be obtained from eligible subjects.

The Principal Investigator will discuss the study at length with the subject. A printed consent form will be supplied, and the subject will be given time to consider his/her decision. The subject will be encouraged to ask further questions about the study to the Investigator or the clinical specialist. Should a subject decide to participate they will be invited to sign the study consent form.

Inclusion Criteria
To be eligible to participate in this trial, subjects must meet all of the following criteria:

1. Diagnosis of sickle cell disease with genotype HbSS, HbS/D^0 thalassemia, HbSD, or HbSO.
2. Age 18-45 years (35-45-year-old patients must be followed at BCH).
3. Receiving regularly-scheduled blood transfusions or exchange transfusions as part of existing medical care.

4. Adequate hematologic parameters including:
    a. White blood cell (WBC) count within the range of 2.5 – 25.0 x 10^9 /L
    b. Hemoglobin within the range of 7 – 11 g/dL
    c. Platelet count within the range of 150 – 700 x 10^9 /L
5. Adequate organ function and performance status:
    a. Karnofsky performance status ≥70%
    b. Serum creatinine </= 1.5 times the upper limit of normal for age, and calculated creatinine clearance or GFR >/= 60 mL/min/1.73 m2.
6. Adequate venous access or an existing venous access device.

Exclusion Criteria
Subjects meeting any of the following criteria will be excluded from the study:

1. Subjects who have uncontrolled illness including, but not limited to:
    a. Ongoing or active infection
    b. Emergency room admission or hospitalization for SCD-related reason in the past 30 days
    c. Major surgery in the past 30 days
    d. Medical/psychiatric illness/social situations that would limit compliance with study requirements as determined by the treating physician.
2. Known myelodysplasia of the bone marrow or abnormal bone marrow cytogenetics.
3. Receipt of an investigational study drug or procedure within 90 days of study enrollment.
4. Pregnant or breastfeeding.
5. Known acute hepatitis or evidence of moderate or severe portal fibrosis or cirrhosis on prior biopsy.
6. Known left ventricular ejection fraction <40%
7. Known DLCO (corrected for hemoglobin), FEV1, and/or FVC < 50% of predicted.
8. ALT or AST > 2.5 X upper limit of normal or direct bilirubin > 2.0 mg/dL.
9. On hydroxurea treatment
10. Requires placement of central line for apheresis

Off study criteria
Subjects will be considered off study if any of the following conditions are met:

1. the patient was not able to donate stem cells.
2. the patient withdraws consent for data submission;
3. completion of the 7-day follow-up period;
4. lost to follow-up prior to the end of the 7-day follow-up period; or,
5. death prior to the end of the 7-day follow-up period.

(3) Description of Study Procedures

Baseline studies
Up to 30 days prior to collection the subject will be approached and if consented, undergo laboratory testing, history, and physical exam.

Pre-plerixafor exchange or simple red cell transfusion
Between Days -7 and -2 prior to apheresis, the subject will undergo an exchange or simple transfusion per their standard of care. This transfusion will be timed in accordance with the patient's existing chronic transfusion regimen. Labs will be drawn before the transfusion, including CBC, differential, reticulocyte count, beta-HCG, and type and screen and a hemoglobin electrophoresis. A transfusion will be performed with post-transfusion hemoglobin electrophoresis confirming a percentage of HbS of </= 40%. A CBC and peripheral CD34+ cell count will be drawn at the end of the procedure.

Admission and Day -1 Blood Sampling
On Day -1, the subject will be admitted to the Hematology service at Boston Children's Hospital. Confirmation of available, compatible units of packed red blood cells will be confirmed prior to administration of plerixafor (for use in case of an unexpected acute clinical need for transfusion). Labs will be drawn, including CBC, differential, type and screen, and peripheral CD34+ cell count. Prior to administration of plerixafor, the following lab results will be confirmed: percentage HbS ≤ 40% in post transfusion hemoglobin electrophoresis (within 1 week of admission), ANC ≥ 1000 cells/uL and platelet count ≥ 150 K/uL on admission. The patient will be hydrated with 0.9% NS until apheresis is discontinued.

Plerixafor administration, post-plerixafor labs and apheresis
On Day 0, the subject will receive a single dose of subcutaneous plerixafor at 240 mcg/kg. Prior to apheresis, labs will be drawn, including CBC, differential, and peripheral CD34+ count. A normal platelet count of ≥ 150 K/uL will be confirmed prior to proceeding with apheresis, starting 2 hours after plerixafor dose. If the subject does not already have central venous access, access for apheresis will be obtained peripherally using 2 large bore 16g needles, and blood will be processed in the cell separator. Blood within the instrument will receive acid citrate dextrose formula A (ACD-A) (3%) at a rate of 1 mL/min/L of total blood volume, which is the standard apheresis dose. 2 grams of calcium gluconate is infused over the course of the procedure to prevent hypocalcemia associated with citrate administration. Peripheral CD34+ count will be checked every 2 hours during the apheresis procedure. Standard local apheresis protocol will be followed to determine cessation of apheresis for any reason. Vital signs are monitored every 15 minutes while on the instrument. The amount of blood processed will be 3-4 blood volumes, a standard range for volunteer donors and not as long as those who were on our previous study. A CBC and Diff and CD34 counts are drawn after apheresis is performed. The subject will remain observed for up to one hour after cessation of apheresis with discharge from the Hematology service after they meet standard clinical discharge criteria and by laboratory check of the post procedure CBC.

Disbursement and Storage of collected cells
The collection product will undergo CD34+ selection in The Connell and O'Reilly Families Cell Manipulation Core Facility (CMCF) at the DFCI, which is part of our GMP Facility. Potential future use could include research use by other BCH investigators or collaborators (academic or Beam Therapeutics) or to the Cure Sickle Cell consortium. There will be no cells stored for

therapeutic use to the volunteer. All research performed on the cells collected in this study will be directed toward sickle cell disease science.

Outpatient follow-up
On Day + 1 after apheresis, the study team will communicate with the subject via phone to inquire about any symptoms experienced. On Day +7, the subject will again be contacted to check for any unexpected symptoms. The hematologist normally following the volunteer will be informed of the results of the laboratory tests.

(4) Outcome of Collection

Primary outcome:
1. Feasibility will be estimated by whether apheresis collection of a minimum of 0.5×10^6 CD34+ cells/kg is successful.

Secondary outcome:
1. Pre and post-apheresis peripheral CD34+ cell concentration to examine mobilization. Efficiency of collection will be calculated and collected cell characteristics will be assessed.

(5) Data Collection Methods, Assessments, Interventions and Schedule
No identifying data will be collected about the donor. Investigators will receive the cells and they understand that they will not receive any identifying information about the donors.

5.1 Assessments of safety
A full physical examination (including chest auscultation, anthropometric measurement, body temperature, heart rate, respiratory rate and blood pressure measurement) will be carried out at baseline, admission, during the study admission, and at each follow-up visit to evaluate the subject's condition. (See Appendix A – Schedule of Events). Safety will be assessed through evaluation of adverse reaction after collection and adverse reactions observed by the investigator and research team or reported by the subject during the study period (see Section D: Adverse Event Reporting Procedures).

5.2 Laboratory assessments for primary endpoints
At the timepoints outlined in Appendix A, blood will be collected in EDTA tubes to use for a complete blood count and WBC differential, peripheral CD34+ count. CD34+ cells will be enumerated using a single-platform, 2-color assay performed on a Beckman-Coulter XL flow cytometer.

5.3 Laboratory assessments for exploratory research endpoints
Examples of lab assays performed on the samples of cells collected:

a) Immunotyping of CD34+ cells isolated from plerixafor-mobilized peripheral blood
b) Clonogenic assays will be performed, including enumeration of granulocyte-macrophage colony-forming units (CFU-GMs) and erythroid burst-forming units (BFU-Es);
c) Transplantation into immunodeficient mice (20-24 weeks of follow up, engraftment and differentiation in PB, BM, Spl, Thy)
d) Lentiviral transduction with large scale LVs intended for use in our planned upcoming gene therapy trial; optimization of transduction in bags and measurement of VCN, transduction efficiency, HbF expression, clonogenic potential, immunodeficient mice repopulation and differentiation, and maintenance of LV in chimeric mice.

e) Gene editing and assessment of the in vitro and in vivo phenotype of edited cells.

Research investigations above will be performed with cells collected in research labs within Boston Children's Hospital (including the Bauer and Williams labs), colleagues at Beam Therapeutics, or the NIH as part of the Cure Sickle Cell initiative, all for research purposes.

5.4 Assessments of eligibility to collect cells

Each subject will undergo a standard set of laboratory assessments that are performed for any stem cell donor in our institution (autologous, sibling, or unrelated). These include: HIV-1 and 2 antibody, HIV NAT, HTLV-I and II antibody, West Nile virus NAT (seasonal as appropriate), Hep B surface antigen, Hep B core antibody, HBV NAT, HCV NAT, syphilis, CMV antibody, Chagas disease. Any positive results will require PI evaluation to assess for suitability of enrollment. In addition, a standard transplant donor questionnaire will be required.

(6) Safety Issues and Risks

6.1 Plerixafor administration

Adverse effects associated with plerixafor have been mild and transient, including headache, erythema and stinging at injection site, perioral paresthesias, nausea, and sensation of abdominal distension[12]. Hematologic effects observed have included leukocytosis and thrombocytopenia. The most common adverse reactions (>/= 10%) reported in patients who received plerixafor in conjunction with G-CSF regardless of causality and more frequent with plerixafor than placebo during HSC mobilization and apheresis were diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting (drug label information).

6.2 Apheresis

The apheresis procedure lasts from three to four hours and has been shown to be extremely safe both in adults and in children. Adverse reactions to apheresis procedures are rare, although vasovagal episodes related to needle insertions or transient volume loss can occur. The former reaction is prevented by lying down and if necessary, fluid administration. Paresthesia or tingling can also sometimes occur due to lowering of calcium levels by the citrate anticoagulant. This is readily relieved by slowing the rate or temporarily interrupting the anticoagulant infusion. The precautions taken to minimize pain, hematoma, risk of infection at the needle site and vasovagal fainting are outlined in the venipuncture section. Leukapheresis can be associated with some loss of red blood cells and plasma equivalent to 100ml of whole blood though every effort will be made to keep this volume as low as possible. This is a routine procedure with minimum side effects or complications. Potential complications include formation of hematoma at the site and a small risk of infection as with any intravenous catheter or access of a central line.

6.3 Risks of Phlebotomy

Risks associated with a blood draw may include minor discomfort, bruising, fainting, and infection. When possible, we will draw blood at the time of a clinically-indicated procedure to reduce the number of needle sticks.

(7) Costs and Compensation

Costs: For mobilized PBSC collection, The PI will be responsible for all costs related to the administration of filgrastim, plerixafor, laboratory tests, apheresis, processing, and storage of the collected blood cells. Funding sources for the collection include Federal NIH funding,

Department of Lab Medicine funds, and industry funding including Beam Therapeutics. This will include costs of obtaining medications, needles, needle disposal boxes, and syringes for administration of the medication, medications used to treat adverse effects of intravenous access, plerixafor, apheresis, and any ex vivo processing. Volunteer donors will not be charged for any costs related to the donation of peripheral blood stem cells.

Compensation: Donors consented for mobilized PBSCs will be compensated up to $2000 for participation in the study. Individuals who enroll, complete informed consent, but are not able to donate PBSCs will receive a pro-rated compensation of $200 for their participation. If individuals complete their visit for transfusion and labs, they will receive an additional $200. Once the patient is admitted and plerixafor administrated, they will receive $1000 in compensation. All payments associated with participation in study visits will be made using a service called ClinCard® by the company Greenphire, www.greenphire.com,

D. ADVERSE EVENT CRITERIA AND REPORTING PROCEDURES

(1) Recording adverse events

The Study PI John Manis will review all adverse events graded according to the criteria specified by the National Cancer Institute's (NCI) Common Toxicity Criteria (CTC, Version 4.0) after each collection. All grade III-IV Adverse Events will be monitored from the time of informed consent to a period of 30 days after collection and reviewed with the Co-investigators Dr.'s Dan Bauer, Erica Esrick, and Matt Heeney within 30 days.

It is the responsibility of the treating physician to document all adverse experiences in the patient chart. At each assessment, adverse experiences will be evaluated, and a detailed description of the event will be documented on an adverse event case report form that will include:

- Description of event
- Onset date and time
- Duration of event
- Severity of event (Grade I-IV based on NCI CTC, Version 4.0)
- Relationship to study: Possibly related, Probably related, Definitely related
- Classification of event: Expected, Unexpected
- Action taken: None, Collection interrupted, Collection discontinued
- Patient outcome to date: Recovered, Recovered with sequelae, Not yet recovered, Death

(2) Reporting requirements

Serious Adverse Events:
For this protocol, a serious adverse event is defined as any untoward medical occurrence attributable to a protocol related procedure that suggests a significant hazard, contraindication, side effect or precaution. In addition, for the purposes of this study, death from any cause while on this protocol will be considered a serious adverse event. Thus, this includes any experience that:

1. Results in death: for this study, this is defined as death from any cause while a subject is on this protocol.
2. Is life-threatening: this is defined as any >Grade 4, (NCI Common Toxicity Criteria) life-threatening event which is possibly, probably or definitely attributable to the investigational agent
3. Is permanently disabling

4. Results in persistent or significant disability/incapacity
5. Is a new malignancy
6. Requires unanticipated in-patient hospitalization or prolongation of existing hospitalization
7. Leads to a congenital anomaly or birth defect
8. Is an event that required intervention to prevent impairment or damage

All serious adverse events that occur during the time the patient is on this study, whether or not related to the study, must be reported by the Principal and the IRB within 24 hours of knowledge of the occurrence.

Protocol Deviations and Exceptions:
Major protocol deviations related to this study but not to patient care must also be reported to the PI. All minor deviations and exceptions will be reported in the annual report to the IRB

Broad guidelines defining major protocol deviation or exception are below. Events that actually or potentially:

i) impact subject safety, welfare or rights
ii) alter the risks or the benefit to the subject in a more significant, serious or negative way
iii) impact the integrity of the data
iv) affect a subject's willingness

(3) Data Safety Monitoring Plan

In this research study we want to learn more about collections of peripheral blood stem cells from patients with sickle cell disease (SCD). The goal of this research study is to collect peripheral blood stem cells from people with sickle cell disease (SCD) and distribute those cells to research laboratories because we hope it will help make possible a new kind of SCD treatment called gene therapy. Currently, bone marrow transplantation (BMT) is the only treatment that has the potential to cure SCD. Doctors here at Boston Children's Hospital (BCH) and at other locations are beginning research trials to find out if another treatment called gene therapy can be used instead of BMT. One of the most important parts of gene therapy is temporarily taking out some of the patient's early hematopoietic stem cells (HSCs), so that these cells can be "fixed" using gene therapy, and then put back into the patient (HSCs are discussed in more detail below). Doctors are working on finding out the best way to collect/obtain these HSCs in a way that is safe and also collects a high enough number of cells. Our study will allow us to understand how to improve the collection of stem cells in sickle cell patients and to generate a source of stem cells for labs working on SCD. The repository of SCD cells will help meet the needs of other workers in the field of novel cell therapies for this disease.
We will collect peripheral HSPCs from SCD patients by apheresis. Subjects will be administered the standard plerixafor mobilization dose of 240 mcg/kg and hydrated in the hospital overnight, with subsequent apheresis performed for 3-4 blood volumes. Patients will be observed to ensure that they tolerated their apheresis collection without adverse events and discharged. A follow up call will be made the following day after discharge to assess symptoms and detect adverse events. The Data and Safety Monitoring Plan (DSMP) outlined below for OT2 HL152800-01 will adhere to the protocol approved by the IRB- P00034825.

OVERSIGHT RESPONSIBILITIES
Oversight and monitoring of the trial is provided by the Principal Investigator (PI) Dr. John Manis, who will consult and meet with and Dr.'s Dan Bauer, Erica Esrick, and Matt Heeney ("co-investigators"). All AE and monitoring reports will be discussed with the co-investigators. Monitoring reports will be sent to the IRB for review. The principal investigator is responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency below. During the review process the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. The principal investigator or the BCH IRB have the authority to stop or suspend the study or require modifications.

MONITORING PROCEDURES

a) Dr. Manis assures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the study is conducted according to the IRB-approved research plan. All patients that are approached and receive any study materials will be documented and reported in the semi-annual monitoring report.

b) Accrual, all subjects approached, consented including withdrawals and lost to follow up will be tracked. Confidentiality will be ensured by using a coded master subject list with the code list kept securely by the PI. All identifying information will be kept on the secure BCH server with access only by the PI. The database will be secured with password protection. Electronic communication with collaborators will involve only unidentifiable information. AE reports and annual summaries will not include subject- or group-identifiable material. Each report will only include the identification code.

c) All patient data for intake, lab values and demographics will be reviewed in real time and also at the end of collection. This will be done by the PI and independently by the study research coordinators. The BCH Transfusion Medicine cell therapy quality specialist will be responsible for keeping protocols and data forms up to date as approved. Quality metrics for accrual, lack of accrual, missing data points and adverse events will be tracked semi-annually. Abnormal laboratory values will be defined by parameters outside the normal range as reported in the ERM, or outside the range of the pre-admission values. The semi-annual Report will be compiled and will include a list and summary of AEs and will state whether AE rates are consistent with pre-study assumptions, reason for dropouts from the study, whether participants met entry criteria and whether conditions whereby the study might be terminated prematurely were met.

d) Study data are accessible at all times for the PI and co-investigators to review. The PI and co-investigators review study conduct after each patient and semi-annually when the monitoring report is generated. The PI and co-investigators review AEs individually in real-time after each patient is collected and in aggregate on a semi-annual basis. The PI and co-investigators review serious adverse events (SAEs), within 24 hours of being notified. All SAE's will be reported to the IRB and to the NHLBI.

e) The study will be stopped for any SAE and will be re-opened only after review by the IRB. All study terminations will be reported to the NHLBI. The study will be temporarily suspended for any recurrent AE's that occur more than three times during the trial period to allow for review of the recurrent event and assess causation. This study will be stopped if: a) the intervention is associated with serious adverse effects b) difficulty in study recruitment or retention c) any new information becomes available during the trial that necessitates stopping the trial or d) other situations occur that might warrant stopping the trial. During the funding of this study, any action by the IRB or one of the study investigators that results in a temporary or permanent suspension of the study will be reported to the NHLBI Program Official within 1 business day of notification. Any amendments or changes to the protocol will be reported to the NHLBI within 5 business days.

f) The PI and Co-PI's will report any conflict of interest as per the BCH hospital policy. All COI's in direct conflict with the trial will be reported to the NHLBI and the IRB.

g) The monitoring plan will consist of : Subject accrual (compliance with protocol enrollment criteria), status of all enrolled subjects, adherence data regarding study visits and intervention, AEs and rates including out-of-range lab values, and review of SAE's semi-annually and after each patient is collected. All records will be reviewed. AE's will be reported within 5 business days to the PI and all SAE's will be reported with 24 hours of notification to the PI, Co-PIs and the IRB.

h) Data accuracy will be reviewed by the PI who is responsible for protocol compliance, data collection, documentation and verification.

COLLECTION AND REPORTING OF SAEs AND AEs

For this study, the following standard AE definitions are used:

Adverse event: Any unfavorable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any AE that results in any of the following outcomes:

- Death
- Life-threatening

- Event requiring inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

AEs are graded according to the following scale:
Mild: An experience that is transient and requires no special treatment or intervention. The experience does not generally interfere with usual daily activities, and it does not have a major impact on the patient. This includes transient laboratory test alterations.
Moderate: An experience that is alleviated with simple therapeutic treatments. The experience impacts usual daily activities, and it causes the patient some minor inconvenience. Includes laboratory test alterations indicating injury, but without long-term risk.
Severe: An experience that requires therapeutic intervention. The experience interrupts usual daily activities, and it causes a substantial disruption to the patient's well-being. If hospitalization (or prolongation of hospitalization) is required for treatment it becomes an SAE.
The study uses the following AE attribution scale: AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled definitely unrelated, definitely related, probably related, or possibly related to the study intervention.
Not related: The AE is clearly not related to the study procedures (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).
Possibly related: An event that follows a reasonable temporal sequence from the initiation of study procedures, but that could readily have been produced by a number of other factors.
Related: The AE is clearly related to the study procedures.
AEs are identified during enrollment, hospital admission, during the follow up calls and upon completion of the collection, with all lab values validated within 5 days of collection. After discharge, AEs are assessed at time of study follow-up visits.

MANAGEMENT OF RISKS TO SUBJECTS

Expected AEs
Expected AEs associated with the drugs being used in the study and study procedures include:

- Plerixafor: Adverse effects associated with plerixafor have been mild and transient, including headache, erythema and stinging at injection site, perioral paresthesias, nausea, and sensation of abdominal distension. Hematologic effects observed have included leukocytosis and thrombocytopenia. The most common adverse reactions (greater than or equal to 10%) reported in non-SCD patients who received plerixafor were diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting.
- Intravenous Line Placement: minor discomfort, bruising, fainting, and infection.
- Apheresis: weakness, nausea or feeling faint due to anxiety or decrease in blood volume, tenderness at needle site, localized infection at needle puncture site, temporary tingling or paresthesia due to citrate anticoagulant, death from air infusion

AE Management
We are taking several steps to minimize the chance of negative effects of plerixafor or collection by ensuring that all people in this study have an exchange or simple transfusion prior to plerixafor (to decrease the amount of sickle hemoglobin, and thus decrease the chance of SCD complications, and by observing and monitoring patients in the hospital after the dose of plerixafor. The apheresis procedure lasts from three to four hours and has been shown to be extremely safe both in adults and in children. Adverse reactions to apheresis procedures are rare, although vasovagal episodes related to needle insertions or transient volume loss can occur. The former reaction is prevented by lying down and if necessary, fluid administration. Paresthesia or tingling can also sometimes occur due to lowering of calcium levels by the citrate anticoagulant. This is readily relieved by slowing the rate or temporarily interrupting the anticoagulant infusion. Leukapheresis can be associated with some loss of red blood cells and plasma equivalent to 100ml of whole blood though every effort will be made to keep this volume as low as possible. This is a routine procedure with minimum side effects or complications
Before intravenous line placement, the subject will receive local anesthesia. When possible, we will draw blood at the time of a clinically-indicated procedure to reduce the number of needle sticks.

DATA ANALYSIS PLANS

It is anticipated that data verification will be performed by someone other than the individual originally collecting the data, or by double-data entry and also verified by the PI. A statement reflecting the results of the ongoing data review will be incorporated into the semi-annual report. The PI will document receipt & review of the monitoring report, resolutions and/or corrective actions to findings on the Site Monitoring Log.

PLAN FOR DATA MANAGEMENT

Compliance of regulatory documents and study data accuracy and completeness will be maintained through an internal study team quality assurance process.

Deidentified data regarding characteristics of the subject, cells, and collection procedures may be made available to the investigators and the Cure Sickle Cell stem cell consortium. In order to maintain subject privacy, all CRFs, IMP accountability records, study reports and communications will identify the subject by the assigned unique subject study number. Direct access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process will be permitted for trial-related monitoring and audits by the sponsor, research ethics committee review, and if needed, regulatory inspection(s).

E. DATA MANAGEMENT METHODS

Data handling, record keeping, sample storage

Subjects will be uniquely identified by a study ID number during collection. Only unidentified data will be stored in a separate database (RedCap) on a secure, password protected servers managed by standard policies and procedures. Deidentified data regarding characteristics of the subject, cells, and collection procedures may be made available to the investigators, the Cure Sickle Cell stem cell consortium, or Beam Therapeutics.

F. QUALITY CONTROL METHOD

(1) Sample storage

A record of retained tissue samples will be completed every time a sample is stored. This includes the study specific subject identification number, date sample was stored, and storage location as well as the date the sample was moved or destroyed. No identifiers will be collected with the samples.

(2) Record retention

Essential documents will be retained for a minimum of five years after completion of the study.

(3) Subject confidentiality

In order to maintain subject privacy, all CRFs, IMP accountability records, study reports and communications will identify the subject by the assigned unique subject study number.

Direct access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process will be permitted for trial-related monitoring and audits by the sponsor, research ethics committee review, and if needed, regulatory inspection(s).

(4) Insurance & indemnity

Boston Children's Hospital shall maintain the self-insurance program as approved by the Risk Management Foundation. Boston Children's Hospital is self-insured under CRICO, which has a rating equal to Best's A-.

G. STUDY ORGANIZATION

(1) Boston Children's Hospital

Boston Children's Hospital (BCH) is a leading pediatric institution in the United States. BCH has an active Sickle Cell Program within the Division of Hematology. Hematology patients are admitted to the Hematology service at BCH and jointly managed by the inpatient service.

(2) GMP facility

The GMP facility is used for the purification of CD34+ stem cells for research use. The GMP facility (managed by Dr. Jerome Ritz) is located in the Jimmy Fund Building of the Dana-Farber Cancer Institute and is connected to BCH and managed by the Joint Program in Transfusion Medicine, which supports BCH, Dana-Farber Cancer Institute and the Brigham and Women's Hospital. The Connell and O'Reilly Families Cell Manipulation Core Facility (CMCF) at the DFCI is located in the Jimmy Fund Building-3 (JF-3). Standard operating procedures (SOPs) for CMCF have been approved by the Laboratory Director and Assistant Medical Director and are reviewed at least annually. Specific procedures have been implemented to ensure the integrity of this product. The laboratory has adequate space for the orderly placement of equipment and materials within the facility and to ensure that only one product is processed in a given work space at a time. The manufacturing facility is classified as an ISO 7 cleanroom. Appropriate process and environmental controls are in place to ensure the facility operates well within the guidelines for an ISO Class 7 clean room. The CMCF laboratory at Dana-Farber is fully equipped for processing and cryopreservation of hematopoietic progenitor and other therapeutic cells as well as providing processing for other cellular products. The CMCF laboratory at the DFCI operates under the direction of Jerome Ritz, MD (Laboratory Director) and Sarah Nikiforrow, MD (Assistant Medical Director). The CMCF and their processes have been designed to meet the current good manufacturing practice (cGMP) and current Good Tissue Practice (cGTP) for hematopoietic cell and other cell processing as required by the Food and Drug Administration's Center for Biologics Evaluation and Research (CBER), The Joint Commission and the Foundation for the Accreditation of Cell Therapy (FACT).

(3) Therapeutic Apheresis Unit

The Therapeutic Apheresis (TA) Unit is under the direction of Dr. John Manis. It is located on Farley 4 at Boston Children's Hospital. The TA program provides lifesaving therapies to both inpatients and outpatients referred for these treatments by the Hematology, Oncology, Neurology, Nephrology and Cardiology Programs at BCH. The therapies currently offered are therapeutic plasma exchange, red cell exchange, collection of peripheral blood progenitor cells (PBPC) for transplant, LDL apheresis, therapeutic phlebotomy, white cell depletion, red cell depletion, and photopheresis. The unit has three bed spaces and is budgeted/staffed with 4.0 Registered Nurse FTE.

H. QUALIFICATIONS OF RESPONSIBLE INVESTIGATORS

a. Dr. Erica Esrick, Co-Investigator, is an Instructor of Pediatrics, Harvard Medical School. Dr. Esrick has experience studying fetal hemoglobin induction in the pre-clinical setting, and she cares for patients with sickle cell disease at the BCH Sickle Cell Program. She is the PI of the gene therapy trial at BCH which uses plerixafor for collection of stem cells.
b. Dr. Dan Bauer, Co-Investigator, Assistant Professor of Pediatrics, Harvard Medical School. Dr. Bauer has experience studying fetal hemoglobin induction in the pre-clinical setting, and he cares for patients with sickle cell disease at the BCH Sickle Cell Program.
c. Dr. Matthew Heeney, Co-Investigator, is an Assistant Professor of Pediatrics at Harvard Medical School, the Associate Chief for Hematology, and Director of the Sickle Cell Program in the Division of Hematology/Oncology. He is an active clinician and clinical investigator in sickle cell disease. He is the PI or Co-Investigator for several ongoing multicenter interventional clinical trials in sickle cell disease and was the site PI for the BCH Core of the former NIH sponsored Sickle Cell Disease Clinical Research Network (SCDCRN) and Comprehensive Sickle Center (CSCC) Clinical Trials Consortium.
d. Dr. John Manis, Co-Investigator, is the Associate Medical Director of the Transfusion Medical Service at Boston Children's Hospital. He is responsible for oversight of technical procedures, performance of procedures, staff supervision, administrative operations and quality monitoring activities related to collection of PBPCs. Dr. Manis has extensive experience with clinical trials in both pediatric HSCT, gene therapy and transfusion medicine. Dr. Manis is also responsible for exchange transfusions at BWH/DFCI where there are 10-15 exchange transfusions monthly for SCD.
e. Ms. Colleen H. Dansereau, RN, MSN, CPN is the Manager of the Gene Therapy Program at Boston Children's Hospital. She has extensive experience in Gene Therapy clinical research trial management and clinical research related activities. She is a board-certified pediatric hematology/oncology registered nurse.
f. Dr. Maureen Achebe, Co-Investigator, is the Clinical Director of the Non-Malignant Hematology Clinic at the Dana-Farber Cancer Institute and the Director of the Sickle Cell Program at Brigham and Women's Hospital. She is an Assistant Professor in Medicine at Harvard Medical School. She has extensive training and experience in conducting clinical trials in adults affected by hemoglobinopathies such as sickle cell disease.
g. Ms. Kimberly Ching, Research Associate, Department of Laboratory Medicine. Kimberly Ching is in the laboratory of Dr. John Manis and has experience in managing samples from SCD patients and in organizing the distribution of samples.

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J. APPENDIX A: Schedules of Events

	Baseline[1]	Day -7[2]	Day -1	Day 0	Day +1	Day +7 (+/- 1 day)
ASSESSMENTS						
History and Physical	X	X	X	X		
CBC/differential	X	X,X	X	X,X,X,X		
Hemoglobin electrophoresis		X[3]				
Type and screen	X	X	X			
Creatinine	X					
AST, ALT, and total and direct bilirubin	X					
Peripheral CD34+ count	X		X	X,X,X,X		
Infectious disease marker testing	X					
Beta-HCG	X	X				
Phone assessment					X	X
ACTIONS / PROCEDURES						
Exchange or simple transfusion		X				
Admission to Hematology service			X			
Plerixafor administration				X		
Apheresis collection				XX		
Discharge from Hematology service				X		

[1] Baseline assessment to be completed by day -30
[2] Transfusion can occur between Day-7 and Day-2
[3] Pre- and post-exchange or simple transfusion